To the detergent composition of the present invention, various enzymes can be used in combination with the alkaline protease of the present invention. Examples include hydrolases, oxidases, reductases, transferases, lyases, isomerases, ligases and synthetases. Of these, proteases, cellulases, lipases, keratinases, esterases, cutinases, amylases, pullulanases, pectinases, mannases, glucosidases, glucanases, cholesterol oxidases, peroxidases, laccases and proteases other than the alkaline protease used in the present invention are preferred.

Please replace the paragraph bridging pages 21 and 22 with the following:

Mutation was introduced at random into a protease structural gene of about 2.0 kb including a termination codon by the following manner. First, PCR was conducted using a primer capable of amplifying this 2.0kb. A PCR master mix contained 5 ng of a template DNA, 20 pmoL of a phosphorylated primer, 20 nmoL of each dNTP, 1 μmoL of Tris/HCl (pH 8.3), 5 μmoL of KCl, 0.15 μmoL of $MgCl_2$ and 2.5U TaqDNA polymerase, and its total amount was adjusted to 100 μL. After modification of the template by allowing it to stand at 94°C for 5 minutes, PCR was performed for 30 cycles, each cycle consisting of treatment at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1.5 min. The PCR product was purified by "PCR product purification Kit" (product of Boeringer Manheim), followed by elution in 100 μL of sterile water. With 1 μL of the eluate, second PCR was conducted under conditions similar to those of the first PCR except for the template DNA. After completion of the second PCR, the PCR product was purified in a similar manner to the first PCR, followed by elution in 100 μL of sterile water.

Please replace the paragraph bridging pages 22 and 23 with the following:

The amplified DNA fragment was integrated in a vector by polymerase reaction using "LATaq" produced by Takara Shuzo Co., Ltd. Described specifically, after addition of

5 μL of a buffer for LATaq (a 10-fold concentrate), 8 μL of a dNTP solution and 0.5 μL of LATaq DNA polymerase, and as a template, 20 ng of plasmid pHA64TS (having a protease structural gene linked with an expression vector pHA64) to 35 μL of the purified eluate, the total amount was adjusted to 50 μL. PCR reaction of the resulting liquid was carried out for 30 cycles, each consisting of treatment at 94°C for 1 min, 55°C for 1 min and 72°C for 4 min. By the subsequent ethanol precipitation, the PCR product was collected. This PCR product had a shape of a plasmid having a nick at the 5' prime end of the primer. Ligase reaction by T4 ligase (product of Takara Shuzo Co., Ltd.) was conducted to link this nick portion.

a4

Page 23, please replace the paragraph beginning at line 11 with the following:

By using 10 μL of this ligase reaction mixture, transformation of the *Bacillus subtilis* strain ISW1214 was conduced, whereby about 4×10^5 transformants were obtained. The resulting transformants of the strain ISW1214 were cultured on a skin-milk-containing medium (containing 1% skim milk, 1% bactotrypton, 1% sodium chloride, 0.5% yeast extract, 1.5% agar and 7.5 μg/ml of tetracycline) and halo formation, which was presumed to reflect the protease secretion amount, was observed.

a5

Please replace the paragraph bridging pages 23, 24 and 25 with the following:

The protease active fraction was prepared in the following manner. The transformants obtained in Example 1 was cultured at 30°C for 60 hours on a medium A (3% polypeptone S (product of Nippon Pharmaceutical), 0.5% yeast extract, 1% fish meat extract (product of Wako Pure Chemical Industries, Ltd.), 0.15% dipotassium phosphate, 0.02% magnesium sulfate 7 hydrate, 4% maltose and 7.5 μg/mL of tetracycline). The supernatant of the thus-obtained cultured medium was added with ammonium sulfate to give 90%

a6

saturation, whereby salting-out of protein was caused. The sample obtained by salting-out was dissolved in a 10 mM tris HCl buffer (pH 7.5) containing 2 mM of calcium chloride. The resulting solution was dialyzed overnight against the same buffer by using a dialysis membrane. The fraction in the dialysis membrane was applied to DEAE Bio-Gel A (product of Bio-Rad Laboratories) equilibrated with a 10 mM tris HCl buffer (pH 7.5) containing 2 mM calcium chloride to collect the protease active fraction not adsorbed to the ion-exchanger. This active fraction was applied further to "SP-Toyopearl 550W" (product of Tosoh Corp.) equilibrated with the same buffer, followed by elution with a 0 to 50 mM sodium chloride solution, whereby a protease active fraction was obtained. The resulting fraction was analyzed by SDS-PAGE electrophoresis to confirm that the protease was obtained as substantially uniform protein. The protein concentration was measured in accordance with the method of Lowry, et al. (J. Biol. Chem. **193**, 265-275(1981)) by using bovine serum albumin (product of Bio-Rad Laboratories) as a standard.

a7

Please replace the paragraph bridging pages 30 and 31 with the following:

In 2 mL of a 100 mM borate buffer (pH 10.5) containing 3% of aqueous hydrogen peroxide, a 50 μL portion of each of the protease variants obtained by purification in Example 1 was added. The resulting mixture was allowed to stand at 30°C for 30 minutes. After addition of an adequate amount of catalase (product of Boehringer Manheim) to remove excess hydrogen peroxide, the residual protease activity was measured by the synthetic substrate assay. In FIG. 3, the residual activity after treatment with aqueous hydrogen peroxide is shown relative to the activity before treatment set at 100%.

a8

Please delete the original Sequence Listing from page 32 to page 56 of the specification.

Page 63 (Abstract), after the last line, beginning on the next page, insert the substitute Sequence Listing attached hereto.